An insulator-based (electrodeless) dielectrophoretic concentrator for microbes in water

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Abstract

Dielectrophoresis (DEP), the motion of a particle caused by an applied electric field gradient, can concentrate microorganisms non-destructively. In insulator-based dielectrophoresis (iDEP) insulating microstructures produce non-uniform electric fields to drive DEP in microsystems. This article describes the performance of an iDEP device in removing and concentrating bacterial cells, spores and viruses while operated with a DC applied electric field and pressure gradient. Such a device can selectively trap particles when dielectrophoresis overcomes electrokinesis or advection. The dielectrophoretic trapping behavior of labeled microorganisms in a glass-etched iDEP device was observed over a wide range of DC applied electric fields. When fields higher than a particle-specific threshold are applied, particles are reversibly trapped in the device. Experiments with *Bacillus subtilis* spores and the Tobacco Mosaic Virus (TMV) exhibited higher trapping thresholds than those of bacterial cells. The iDEP device was characterized in terms of concentration factor and removal efficiency. Under the experimental conditions used in this study with an initial dilution of 1×10^5 cells/ml, concentration factors of the order of $3000 \times$ and removal efficiencies approaching 100% were observed with *Escherichia coli* cells. These results are the first characterization of an iDEP device for the concentration and removal of microbes in water.
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1. Introduction

World-wide, drinking-water-borne pathogens kill more than 2.5 million people a year (WHO, 2004). Efficient devices that detect microbes in water can help

mitigate this problem, but analytical instruments are hindered by the extreme dilution of these microbes. To be effective, these instruments require significant sample concentration. This article describes the performance of a novel prototypical device that employs insulator-based dielectrophoresis (iDEP) for selectively concentrating pathogens from dilute samples. Conventional sample preparation methods such as mechanical filtration involve a lengthy culture step

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to obtain concentrated samples for identification/ analysis (Greenberg et al., 1992). Other methods for the separation of microorganisms, such as electrophoresis, have the disadvantage that the microbes are separated based on their characteristic charge-to-mass ratio, which is not generally sufficiently selective and prone to variation in different chemical environments (Armstrong et al., 1999). Dielectrophoresis (DEP) provides an attractive alternative to conventional methods because of its ability to concentrate and separate microorganisms in a selective, rapid, and reversible manner (Cummings and Singh, 2003; Pohl, 1951; Jones, 1995; Pohl, 1978).

DEP is the motion of a particle due to the unbalanced force of a non-uniform electric field on the particle's induced dipole moment: one "end" of the dipole is in a weaker field than the other, causing the particle to be pulled electrostatically along the electric field gradient (Müller et al., 1996). The dielectrophoretic force acting on an isolated spherical particle can be represented as

$$F_{DEP} = 2\pi\varepsilon_0\varepsilon_m r^3 f \nabla E^2 \qquad (1)$$

where ε_0 is the permittivity of free space, ε_m is the relative permittivity of the suspending medium, r is the radius of the particle, E is the local electric field, and f is the Clausius-Mossotti (CM) factor

$$f = \left[\frac{\tilde{\sigma}_p - \tilde{\sigma}_m}{\tilde{\sigma}_p + 2\tilde{\sigma}_m}\right] \qquad (2)$$

where $\tilde{\sigma}_p$ and $\tilde{\sigma}_m$ are the complex conductivities of the particle and the medium, respectively (Jones, 1995; Pohl, 1951). The complex conductivity is related to the real conductivity and dielectric constant by $\tilde{\sigma} = \sigma + i\omega\varepsilon$ and ω is the angular frequency of the applied electric field. For frequencies below 100 kHz or when DC electric fields are applied, the imaginary part of the complex conductivity can generally be neglected (Markx et al., 1994b; Van Den Wal et al., 1997). In the present study, only DC electric fields were used.

To produce the non-uniform fields required to drive DEP, the most common approach is to apply purely AC electric fields through microelectrodes (Washizu and Kurosawa, 1990). Some DEP studies have been focused on the separation of bacterial cells

(Markx et al., 1996, 1994a; Pethig and Markx, 1997; Suehiro et al., 2003a; Li and Bashir, 2002; Suehiro et al., 2003b). Other studies have focused on the dielectrophoretic separation of yeast cells, viruses and parasites (Hughes et al., 2001, 1998; Morgan and Green, 1997; Green et al., 1997; Betts, 1995; Quinn et al., 1996; Betts and Brown, 1999; Pohl and Hawk, 1966; Crane and Pohl, 1968; Pohl and Crane, 1971; Markx et al., 1994b; Medoro et al., 2002; Müller et al., 1996; Schnelle et al., 1996; Suehiro et al., 2003c). The development of micro-fabrication techniques has enabled the construction of micro-electrode arrays (Malyan and Balachandran, 2001; Betts and Brown, 1999). However, microelectrode array-based DEP systems face application-limiting issues, such as the degradation of microelectrodes and decay of the electric field above the planar electrode array, which reduces trapping efficiency (Chou and Zenhausern, 2003).

Insulator-based DEP (iDEP), first developed by Masuda et al. and recapitulated by Lee et al., employs spatially non-uniform insulating structures to produce non-uniformities in an electric field generated by remote electrodes (Masuda et al., 1989; Lee et al., 1994). Devices for iDEP can be made purely from insulating materials (e.g., plastics), which can be replicated inexpensively, facilitating high-throughput and large-volume applications. Chou et al. demonstrated iDEP of DNA molecules, *Escherichia coli* cells, and blood cells using insulating structures and AC electric fields (Chou et al., 2002, 2003). Zhou et al. and Suehiro et al. used channels filled with insulating glass beads and AC electric fields for separating and concentrating yeast cells in water (Zhou et al., 2002; Suehiro et al., 2003d).

Cummings and Singh demonstrated experimentally and analytically on-chip iDEP with DC electric fields using arrays of insulating posts inside a microchannel to trap polystyrene particles (Cummings and Singh, 2000, 2003; Cummings, 2002). More recently, we have demonstrated that iDEP can be used to selectively trap and concentrate both live and dead *E. coli* cells using microchannels containing arrays of circular insulating posts (Lapizco-Encinas et al., 2004a). The separation between live and dead cells arose from differences between the membrane conductivities of the two classes of cells. When a cell

dies, the cell membrane becomes permeable, and its conductivity can increase up to ~10 μS/cm; whereas the conductivity of the membrane of a live cell tends to be ~10^{-3} μS/cm (Pethig and Markx, 1997). These differences in conductivity dramatically change the Clausius-Mossotti factor Eq. (2), producing significantly different dielectrophoretic trapping thresholds for the live and dead particles. While both exhibited negative DEP, the lower trapping threshold (defined as the minimum voltage applied that achieves trapping) of the live cells allowed their selective collection, demonstrating the potential of iDEP for rapid cell viability analysis (Lapizco-Encinas et al., 2004a).

We have also reported the dielectrophoretic separation between different species of live bacterial cells (Lapizco-Encinas et al., 2004b). In this case, parameters other than membrane conductivity play an important role in the separation process. These parameters include cell size, cell shape, and other morphological characteristics of the cells, such as the presence of a flagellum. While the theory is not yet complete enough to predict the relative trapping thresholds of different bacteria, we empirically demonstrated that these thresholds are typically significant enough between species of bacteria to allow selective collection. The threshold applied electric field required to achieve dielectrophoretic trapping of the four species of bacteria in the study, from lowest to highest threshold, was *E. coli* < *Bacillus megaterium* < *Bacillus subtilis* < *Bacillus cereus*. These results demonstrate that iDEP can separate similar species of live bacterial cells (Lapizco-Encinas et al., 2004b).

This publication presents the capabilities of iDEP for the concentration and removal of water-borne bacteria, spores and viruses using our micro iDEP device. The dielectrophoretic behavior exhibited by the different microorganisms was a function of the applied electric field and the characteristics of the microorganism, such as size, shape, and conductivity. The performance of the iDEP device was characterized in terms of both concentration factor and removal efficiency. The results obtained illustrate the potential of the iDEP concentrator as a front-end device with significant applications for the screening and analysis of bacteria, spores, and viruses.

2. Materials and methods

2.1. Microorganisms and inert particles

2.1.1. Bacteria

Lyophilized *E. coli* (strain BL21) were obtained from Stratagene (La Jolla, CA). Cell cultures were grown at 37° C in a shaker incubator for 12 h to achieve saturation conditions. A 1 : 20 volumetric dilution of each cell culture was then allowed to grow in Lennox L Broth (LB) into the late log phase to a cell concentration of 6×10^8 cells/ml, verified by OD measurements at 600 nm (Ausubel et al., 2002). Cells were centrifuged at 5000 rpm for 10 min in order to extract the LB and re-suspended in DI water (pH 8) via a vortex mixer. The cells were then labeled with Syto® 11 (green) or Syto® 17 (red) bacterial stains (Molecular Probes, Inc., Eugene, OR) that respectively fluoresce green (excitation/emission 508/527 nm) and red (excitation/emission 621/634 nm). Three microliters of fluorescent nucleic acid stain (5 mM solution in DMSO) was added for every milliliter of cell culture. The cells were then incubated at room temperature for 15 min. The labeled cells were recovered by centrifugation at 5000 rpm for 10 min, washed three times with DI water to remove any excess dye, and re-suspended in DI water to reach the desired cell concentration (typically 6×10^8 cells/ml). For the determination of concentration factor and removal efficiency, a feed concentration on the order of 1×10^5 cells/ml was utilized.

2.1.2. Spores

Spore suspensions of *B. subtilis* (strain ATCC # 6633) were obtained from Raven Biological Laboratories Inc. (Omaha, NE). The spore samples were labeled with Syto® 11 dye, which was added to the samples as received without any further modification. The spore samples were labeled by following the same protocol used with the live bacterial cells; the final concentration of the labeled spores was 1×10^9 spores/ml. The labeled spore solutions were then added to the inlet reservoir in the flow manifold via pipette, resulting in a spore concentration of approximately 2×10^7 spores/ml in the microchannel liquid reservoir.

2.1.3. Virus

Tobacco Mosaic Virus (TMV) samples (ATCC # PV-135) were obtained from ATCC (Manassas, VA).

The sample of TMV was received purified at a concentration of 2.0 mg virions/ml. TMV was labeled with the fluorescent dye Syto® 11 (Molecular Probes, Inc., Eugene, OR). This dye will cause virions to fluoresce green. The TMV sample was diluted from 0.5 to 1.5 ml by adding 0.001 N carbonate buffer and 5 µl of dye and then incubated at room temperature for 15 min. The TMV samples were concentrated by centrifugation at 14,000 rpm for 30 min, washed three times with DI water to remove any excess dye, and then re-suspended in carbonate buffer to the desired virus concentration (typically 0.66 mg virions/ml). Samples of TMV were then used directly or mixed, and then 20 µl of this sample was added to the inlet reservoir in the flow manifold via pipette, resulting in a virus concentration of approximately 0.1 mg virions/ml in the microchannel liquid reservoir.

2.1.4. Inert particles

Carboxylate-modified polystyrene microspheres, *FluoSpheres*™, (Molecular Probes, Eugene, OR) having a density of 1.05 mg/mm^3 and diameter of 200 nm were utilized at 1:100 dilutions.

2.2. Apparatus

A schematic diagram of the equipment is shown in Fig. 1a. Experiments are conducted in a microfluidic chip consisting of eight patterned channels isotropically etched in glass. The chip is reversibly sealed to a PDMS flow manifold via a vacuum chuck. The manifold provides 16 open reservoirs,

and each reservoir has a volume of 0.1 ml. The manifold and chips are placed directly on an inverted epifluorescence microscope, model IX-70 (Olympus, Napa, CA). Different sets of fluorescence filters are employed: Chroma 51006, Chroma 51004 (Chroma Technologies Corp, Brattleboro, VT) and Olympus 41012 (Olympus, Napa, CA). A high-voltage power supply (Stanford Research Systems, PS350, Palo Alto, CA) is used to apply electric fields to the microsystem via 0.508 mm diameter platinum-wire electrodes (Omega Engineering INC., Stanford, CT) in the fluid reservoirs. Sequences of fluorescent images of the cells are recorded using a MacroFire digital camera (Optronics, Goleta, CA).

2.3. Microfluidic circuit fabrication

The microchips were fabricated from Schott D263 glass wafers (100 mm diameter, 1.1 mm thick, S. I. Howard Glass Company, Worcester, MA) using standard photolithography, wet etch, and bonding techniques. The microfluidic chip contains eight independent microchannels that are 10 µm deep. Each microchannel is straddled by two liquid reservoirs that have an approximate diameter of 1 mm and a depth of 1 mm. The distance between the reservoirs is 10.2 mm; the post-area is located in the middle of the microchannel, 2.9 mm from each via (Fig. 1b). The arrays of insulating structures are circular posts with 200-µm diameters spaced 250 µm center-to-center. The insulating posts traverse the entire depth of the microchannel.
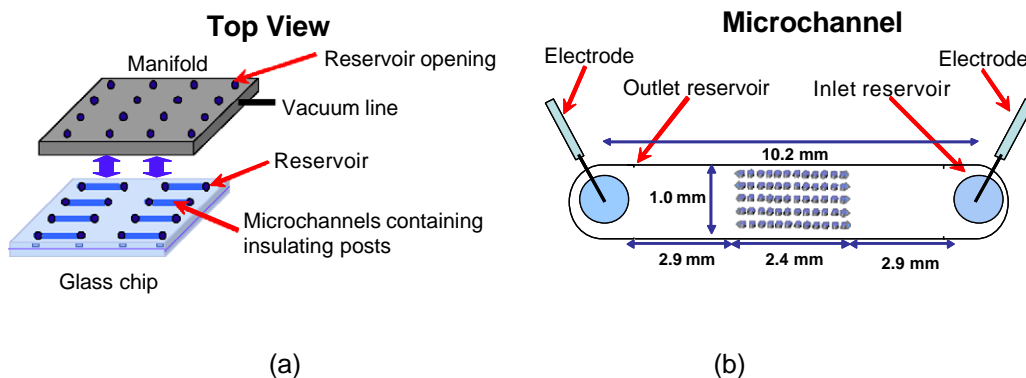

Fig. 1. Schematic representation of (a) flow manifold and glass chip and (b) microchannel containing the array of insulating posts.

2.4. Experiment preparation

The ports in the chip were aligned with the flow manifold (Fig. 1), and the channel and corresponding reservoirs were filled with a background solution consisting of DI water, NaOH, and KCl. The pH of this solution was adjusted to a value of between 7.5–8.0 by adding 0.01 M NaOH. The conductivity of this solution was adjusted to a value of 10 or 20 μS/cm by adding 0.01 M KCl. A sample of labeled microorganisms was introduced at the inlet reservoir. Electrodes were placed at the inlet and outlet reservoir, and an electric field was applied across the 10.2-mm long microchannel containing the post array. The dielectrophoretic behavior of the microorganisms was recorded using the microscope and video camera.

2.5. Determination of concentration factor and removal efficiency

The concentration factor and removal efficiency of the iDEP systems were evaluated by performing experiments where *E. coli* cells with an initial dilution of 1×10^5 cells/ml were concentrated and released. The experiments consisted of three parts:

(a) A low pressure-driven flow (100 Pa), from the inlet to the outlet, was applied by using custom-made liquid reservoirs at each end of the microchannel; and the number of cells passing through the post array was evaluated using fluorescence microscopy.
(b) An electric field was applied for a specified period of time (1 or 2 min) and the *E. coli* cells were dielectrophoretically trapped in the post array; the outlet of the post array was observed in order to determine the number of cells that are able to escape through the post array while the electric field is being applied.
(c) The *E. coli* cells were released from the dielectrophoretic traps as a plug of cells; the concentration of this plug of cells was determined at the outlet of the post array.

For each of these three stages, a 250-frame randomly selected movie was recorded at the outlet of the array. The rate at which particles flow through the device was estimated by counting the number of cells that pass through the outlet during each 250-frame movie. The flow rate of the concentrated plug of cells was determined by counting the number of cells in the movie frame containing the highest population of cells. By calculating the particle flow rate at these particular instances, removal efficiencies and concentration factors were calculated as follows:

$$RE = \left(\frac{CB - CD}{CB} \right) 100\% \tag{3}$$

$$CF = \frac{CP}{CB} \tag{4}$$

where RE is the removal efficiency, CB is the rate at which cells flow past the outlet before the electric field is applied, CD is the rate at which cells flow past the outlet while the electric field is being applied, CF is the concentration factor, and CP is the flow rate of the released plug of concentrated cells as they elute from the post array.

3. Results and discussion

Dielectrophoretic concentration of *B. subtilis* spores and TMV was studied using the iDEP device. In addition, preliminary experiments to characterize device performance in terms of concentration factor and removal efficiency were carried out using *E. Coli*. The results obtained are presented below.

3.1. Dielectrophoretic separations of spores

Spores are very important in water analysis because they are more resistant to traditional water treatments than their respective vegetative cellular forms. Fig. 2a shows fluorescently labeled *B. subtilis* spores collecting in the iDEP device while a mean field of 2000 V/cm was applied. As it can be seen, significant spore concentration is achieved. Fig. 2b shows the release of the spores upon removal of the electric field, demonstrating the reversibility of dielectrophoretic trapping. We observed that the trapping threshold of spores is much higher than that of vegetative bacteria; this opens the possibility to separate vegetative cells from spores. However, spores require
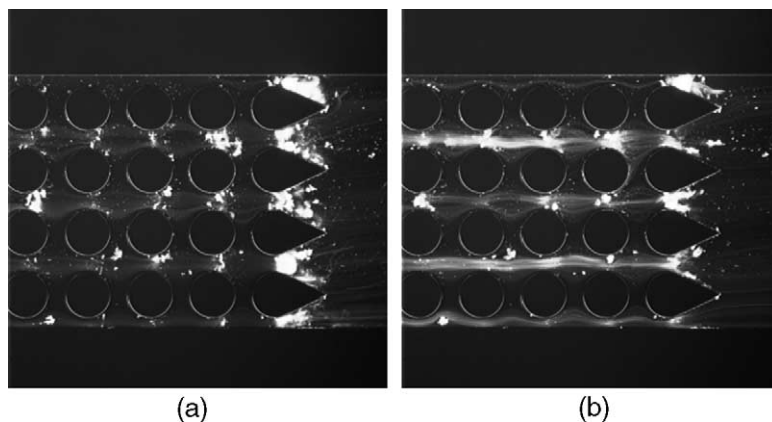
Fig. 2. Trapping and release of *B. subtilis* spores, ×10 magnification. Spore concentration is a concentration of 2×10^7 spores/ml. Spores are labeled green (Syto® 11). In this gray-scale figure, spores appear white. Flow direction is from right to left. The background electrolyte is deionized water, pH=8, σ=20 µS/cm. The circular posts in the array are 10-µm tall, 200 µm in diameter, and on 250-µm centers. The mean applied electric field is 2000 V/cm for (a) DEP trapping and (b) release.

more careful handling than vegetative cells, since spores tend to stick to each other easily, forming clusters that can obstruct the system.

3.2. Dielectrophoretic separation of viruses

The threshold for the dielectrophoretic trapping and concentration of the Tobacco Mosaic Virus (TMV) was generally higher than that of either spores or vegetative cells. Fig. 3 shows the iDEP device collecting fluorescently labeled TMV at a mean applied electric field of 2000 V/cm. Under these experimental conditions, TMV is observed to undergo negative dielectrophoresis. It was also observed that TMV aggregates during the DEP trapping and it releases in clusters. In order to test if the TMV could be selectively trapped against a more-concentrated background, experiments were performed in which a 20 µl sample of 200-nm fluorescently labeled red polystyrene beads at 1:100 dilution were added to the microchannel reservoirs along with the TMV sample. Fig. 4a shows details of TMV trapping between two posts at a mean electric field of 1500 V/cm using a solution only containing TMV. Fig. 4b shows details of TMV trapping in the presence of 200-nm, red-fluorescent, polystyrene particles at a mean applied electric field of 1000 V/cm. The red 200-nm particles are not trapped at this applied field. The electrokinetic mobilities of the beads and viruses were observed to

be nearly identical, thus differences in the trapping behavior are dominated by differences in their dielectrophoretic behavior. These results again demonstrate that DEP can separate particles of similar sizes. Fig. 5 plots the typical threshold mean applied DC electric fields to achieve dielectrophoretic trapping of the different microorganisms in our iDEP device and our suspension liquid. The dielectrophoretic response of the microorganisms studied varies significantly,
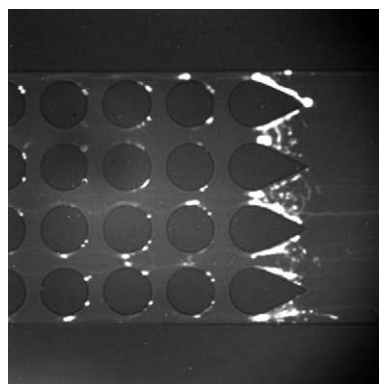

Fig. 3. Trapping of TMV, ×10 magnification. Virus concentration is 0.1 mg of virions/ml. TMV are labeled green (Syto® 11). In this gray-scale figure TMV appears white. Flow direction is from right to left. The background electrolyte is deionized water, pH=8, σ=20 µS/cm. The circular posts in the array are 10-µm tall, 200 µm in diameter, and on 250-µm centers. The mean applied electric field applied is 2000 V/cm.
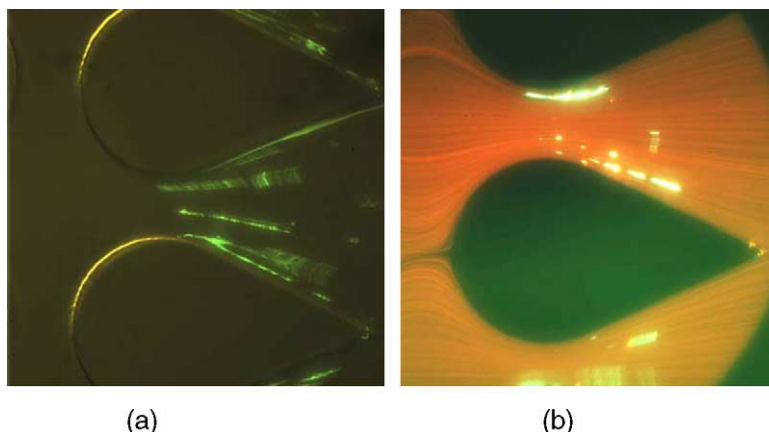
Fig. 4. Trapping of TMV under conditions as described in Fig. 3, unless otherwise stated, ×40 magnification: (a) TMV trapping between two posts at a mean applied field of 1500 V/cm; (b) TMV trapping between two posts in the presence of 200-nm inter particles at a mean applied field of 1000 V/cm. TMV is labeled green and the red background is provided by the presence of 200-nm polystyrene particles at 1:100 dilution.

showing the potential of iDEP for the selective concentration, separation and removal of mixtures of microorganisms.

3.3. Device performance

Fig. 6a and b show the concentration factor and removal efficiency obtained for *E. coli* cells, respectively. The experimental results depicted in Fig. 6 were obtained by applying mean electric fields of 500, 750, and 1000 V/cm for either 1 or 2 min. Three experiments were conducted in each configuration for a total of
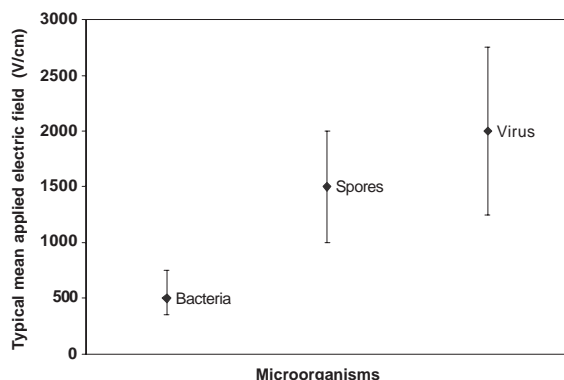

Fig. 5. Typical mean applied electric field (V/cm) required to achieve dielectrophoretic trapping of the different microorganisms studied in our system.
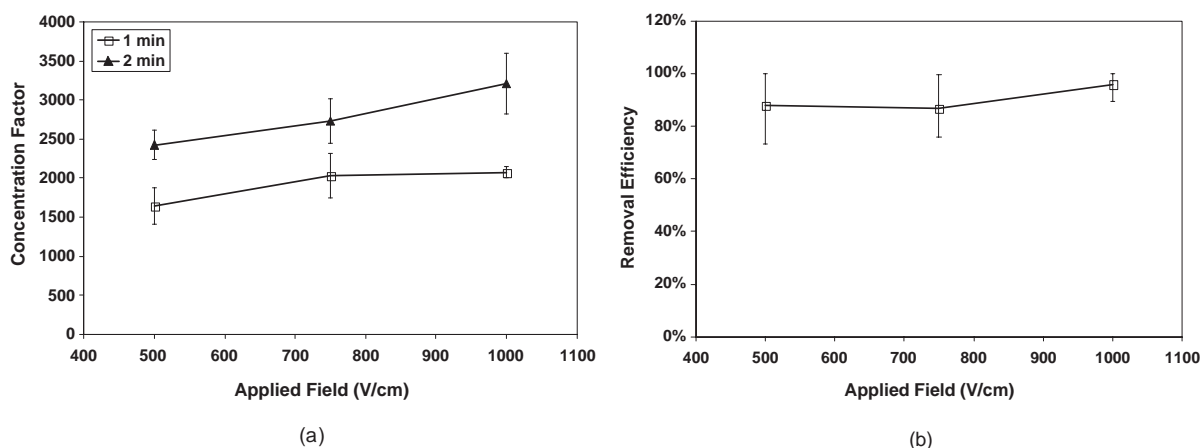
eighteen measurements. Fig. 6a shows concentration factors are all above three orders of magnitude. The initial *E. coli* concentration was increased up to 3200× from an initial dilution of 1×10^5 cells/ml. Fig. 6a shows that the concentration factor increases with the duration of the collection, indicating that the collector has not reached a state of saturation or reached a performance limit in these tests. The concentration factor also increases with increasing applied electric field. These results are expected since, at a given time, the number of cells that have entered the device is proportional to the electric field. Moreover the potential well depth of the dielectrophoretic traps increases with the electric field, so the capacity of the iDEP device increases with increasing field. The concentration factor was measured by counting the particles as they eluted from the post array and it should be noted that the band will become more dispersed further downstream when a pressure-driven flow is applied.

For comparison, Suehiro et al. (Suehiro et al., 2003d) studied a similar system in which dielectric spheres were used as the insulating material to create the non-uniform electric field. A suspension of yeast cells was passed through a chamber containing the insulating spheres, and an electrical field was applied. Cell concentration at the effluent was quantified using a colony counting technique. Suehiro et al. reported a concentration factor of 5, a removal efficiency of 99.999%, and recovery efficiency of 70%.

Fig. 6. Results obtained with *E. coli* cells using an initial dilution of 1×10^5 cells/ml: (a) concentration factor ($n = 3$) and (b) removal efficiency ($n = 6$).

Under the experimental conditions described in this manuscript, the removal efficiency of our micro iDEP device also approached 100%. Fig. 6b shows the average removal efficiency as a function of the applied electric field. Good removal efficiencies were obtained even at the lowest applied electric field (500 V/cm) since this field is significantly higher than the trapping threshold for *E. coli* cells in our system (Lapizco-Encinas et al., 2004b). Fig. 6b shows that the removal efficiency is largely independent of the magnitude of the applied electric field in these experiments. The preliminary results presented in this study were conducted at low initial concentrations to ensure that the experimental conditions were far from saturation to remove particle-to-particle interaction effects and to emulate the particular application we are addressing—water analysis. Future studies will be conducted with varying initial concentrations and operating the devices near saturation, where the removal efficiency should drop precipitously (Davalos et al., 2004). These experimental results on a prototype concentrator show tremendous promise for iDEP in sample concentration for water analysis.

4. Conclusions

An insulator-based dielectrophoretic (iDEP) device has been demonstrated to concentrate microor-ganisms in water. This concentration was achieved by dielectrophoretically trapping particles, which occurs upon applications of an electric field that is larger than a particle-specific threshold. The thresh-old field to trap *B. subtilis* spores was larger than those of vegetative *B. subtilis* cells. This difference allows iDEP devices to separate vegetative cells from spores. The threshold field for trapping Tobac-co Mosaic Virus was observed to be larger than those required to trap bacterial cells or spores. Ad-ditionally, TMV was selectively concentrated against a background of 200-nm polystyrene particles, dem-onstrating the ability of iDEP to separate particles having similar sizes.

The performance of our iDEP concentrator was characterized in terms of concentration factor and removal efficiency. For the experimental conditions described in the manuscript, the concentration of bacterial cells by more than three orders of magni-tude and removal efficiencies approaching 100% were attainable with the iDEP device. These results are particularly encouraging, since the prototype iDEP device had not been engineered specifically to achieve a high concentration performance. Future optimized and fully integrated iDEP designs prom-ise significantly higher concentration factors and will be developed.

Insulator-based DEP has great potential for appli-cations in water analysis and is currently being developed for use as a front-end concentrator for

high-throughput devices that identify microorganisms. Furthermore, iDEP devices can be fabricated from inexpensive insulating materials without the need for thin metal film deposition, which supports the economical fabrication of iDEP concentrators that are scaled to process liters of water, as required for water analysis.

Acknowledgements

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